PATENT COOPERATION TREATY

REC'D 20 OCT 1998
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PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 359292000240	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US97/12253	International filing date *(day/month/year)* 10 JULY 1997	Priority date *(day/month/year)* 10 JULY 1996
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant INTELLIVAX, INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☒ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15 JANUARY 1998	17 SEPTEMBER 1998
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Jeffrey S. Pakrin, Ph.D. Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (January 1994)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US97/12253

I. Basis f the report

1. This report has been drawn on the basis of *(Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments)*:

[X] the international application as originally filed.

[X] the description, pages 1-52 , as originally filed.
pages NONE , filed with the demand.
pages NONE , filed with the letter of _____.
pages _____ , filed with the letter of _____.

[X] the claims, Nos. 1-32 , as originally filed.
Nos. NONE , as amended under Article 19.
Nos. NONE , filed with the demand.
Nos. NONE , filed with the letter of _____.
Nos. _____ , filed with the letter of _____.

[X] the drawings, sheets/~~fig~~ 1-4 , as originally filed.
sheets/~~fig~~ NONE , filed with the demand.
sheets/~~fig~~ NONE , filed with the letter of _____.
sheets/~~fig~~ _____ , filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

[X] the description, pages NONE.

[X] the claims, Nos. NONE.

[X] the drawings, sheets/~~fig~~ NONE.

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the ~~Supplemental Box~~ Additional observations below (Rule 70.2(c)).

4. Additional observations, if necessary:

NONE

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
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III. Non-establishment f opinion with regard t novelty, inventive st p and industrial applicability

The question whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application.

☒ claims Nos. 9 and 24

because:

☐ the said international application, or the said claim Nos. _ relate to the following subject matter which does not require international preliminary examination *(specify)*.

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 9 and 24 are so unclear that no meaningful opinion could be formed *(specify)*.

Applicants have failed to provide a sequence listing in electronic format (e.g., machine readable form) for claims 9 and 24. Pursuant to PCT Rule 13ter.1, these claims have not been examined.

☐ the claims, or said claims Nos. _ are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos. _.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

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V. Reasoned statement under Article 35(2) with regard t novelty, inventive step r industrial applicability; citations and explanations supporting such statement

1.- STATEMENT

Novelty (N)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO
Inventive Step (IS)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO
Industrial Applicability (IA)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO

2. CITATIONS AND EXPLANATIONS

Claims 1-7, 11-14, 17, 19-22, 25, 27, and 28 lack novelty under PCT Article 33(2) as being anticipated by Lowell *et al.* (1988, J. Exp. Med., 167:658-663), hereafter referred to as Lowell *et al.* (1988a). Lowell *et al.* (1988a) teach the preparation of vaccine compositions comprising an antigen with endogenous hydrophobic sequence of between about 3 and about 50 amino acids coupled to an exogenous hydrophobic sequence, which in turn is complexed with a proteosomes to form a proteosomal composition (refer to page 659, first paragraph and Materials and Methods). This teaching also discloses the coupling of lauroyl or pentapeptide (Phe-Leu-Leu-Ala-Val) groups to the antigen of interest to facilitate proteosomal complex formation. Proteosomal complexes were formed in the presence of detergent which was subsequently removed through dialysis. This teaching meets all the limitations of the claimed invention.

Claims 1-7, 11-14, 17, 19-22, 25, 27, and 28 lack novelty under PCT Article 33(2) as being anticipated by Lowell *et al.* (1988, Science, 240:800-802), hereafter referred to as Lowell *et al.* (1988b). Lowell *et al.* (1988b) teach the preparation of vaccine compositions comprising an antigen with endogenous hydrophobic sequence of between about 3 and about 50 amino acids coupled to an exogenous hydrophobic sequence, which in turn is complexed with a proteosomes to form a proteosomal composition (refer to page 659, first paragraph and Materials and Methods). This teaching also discloses the coupling of lauroyl or pentapeptide (Phe-Leu-Leu-Ala-Val) groups to the antigen of interest to facilitate proteosomal complex formation. Proteosomal complexes were formed in the presence of detergent which was subsequently removed through dialysis. Cysteine residues were also added between the antigen and hydrophobic foot to enhance the immunogenic properties of the vaccine composition. This teaching meets all the limitations of the claimed invention.

Claims 8, 10, 15, 16, 18, 23, 26, and 29-32 lack an inventive step under PCT Article 33(3) as being obvious over Lowell *et al.* (1988a, 1988b) in view of VanCott *et al.* (1995) and Levi *et al.* (1995). Lowell *et al.* (1988a) disclose the preparation of (Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(6): A61K 38/00; C07K 1/00; A61K 39/21, 39/385, 45/00 and US Cl.: 530/300, 402, 403; 424/188.1, 193.1, 278.1, 283.1

V. 1. REASONED STATEMENTS:
The report as to Novelty was positive (YES) with respect to claims 8, 10, 15, 16, 18, 23, 26, and 29-32.
The report as to Novelty was negative (NO) with respect to claims 1-7, 11-14, 17, 19-22, 25, 27, and 28.
The report as to Inventive Step was positive (YES) with respect to claims NONE.
The report as to Inventive Step was negative (NO) with respect to claims 1-8, 10-23, and 25-32.
The report as to Industrial Applicability was positive (YES) with respect to claims 1-8, 10-23, and 25-32.
The report as to Industrial Applicability was negative (NO) with respect to claims NONE.

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
vaccine compositions comprising an antigen with endogenous hydrophobic sequence of between about 3 and about 50 amino acids coupled to an exogenous hydrophobic sequence, which in turn is complexed with a proteosomes to form a proteosomal composition (refer to page 659, first paragraph and Materials and Methods). This teaching also discloses the coupling of lauroyl or pentapeptide (Phe-Leu-Leu-Ala-Val) groups to the antigen of interest to facilitate proteosomal complex formation. Proteosomal complexes were formed in the presence of detergent which was subsequently removed through dialysis. This teaching does not specifically describe vaccine compositions comprising HIV gp160 antigens or the administration of said vaccine compositions via intranasal or respiratory routes.

Lowell *et al.* (1988b) teaches the preparation of vaccine compositions comprising an antigen with endogenous hydrophobic sequence of between about 3 and about 50 amino acids coupled to an exogenous hydrophobic sequence, which in turn is complexed with a proteosomes to form a proteosomal composition (refer to page 659, first paragraph and Materials and Methods). This teaching also discloses the coupling of lauroyl or pentapeptide (Phe-Leu-Leu-Ala-Val) groups to the antigen of interest to facilitate proteosomal complex formation. Proteosomal complexes were formed in the presence of detergent which was subsequently removed through dialysis. Cysteine residues were also added between the antigen and hydrophobic foot to enhance the immunogenic properties of the vaccine composition. This teaching also fails to disclose vaccine compositions comprising HIV gp160 antigens or the administration of said vaccine compositions via intranasal or respiratory routes.

VanCott *et al.* (1995) teaches that oligomeric HIV gp160 displays high reactivity toward divergent mAbs and should be included in potential HIV vaccines (see page 103, Abstract and page 115, Discussion). Levi *et al.* (1995) teaches that the intranasal immunization of mammals with proteosomal vaccines confers protection following viral challenge. Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to utilize known immunogens derived from infectious agents, as taught by VanCott *et al.* (1995) and Levi *et al.* (1995), in the proteosomal compositions described by Lowell *et al.* (1988a, 1988b), since this represents an efficient means for generating antigen-specific immune responses. One of ordinary skill in the art would be motivated to utilize different immunization sites (e.g., intranasal) and regimens depending upon the nature of the immune response desired (e.g., mucosal). Finally, one of ordinary skill in the art could employ lyophilization, or other art-recognized methods of vaccine preparation, to make the proteosomal compositions.

---------- NEW CITATIONS ----------

LOWELL et al. Peptides Bound to Proteosomes via Hydrophobic Feet Become Highly Immunogenic Without Adjuvants. J. Exp. Med. February 1988, Vol. 167, pages 658-663, see entire document.

LOWELL et al. Proteosome-Lipopeptide Vaccines: Enhancement of Immunogenicity for Malaria CS Peptides. Science. 06 May 1988, Vol. 240, pages 800-802, see entire document.

VANCOTT et al. Characterization of a Soluble, Oligomeric HIV-1 gp160 Protein as a Potential Immunogen. J. Immunol. Methods. 1995, Vol. 183, pages 103-117, see entire document.

LEVI et al. Intranasal Immunization of Mice Against Influenza with Synthetic Peptides Anchored to Proteosomes. Vaccine. 1995, Vol. 13, No. 14, pages 1353-1359, see entire document.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/30, 15/31, 15/49, A61K 39/39, 39/21 — A3

(11) International Publicati n Number: **WO 98/01558**

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(60) Parent Application or Grant
(63) Related by Continuation
US 60/021,687 (CIP)
Filed on 10 July 1996 (10.07.96)

(71) Applicants *(for all designated States except US)*: INTELLIVAX, INC. [US/US]; 6303 Western Run Drive, Baltimore, MD 21215 (US). HENRY M. JACKSON FOUNDATION [US/US]; Suite 600, 1401 Rockville Pike, Rockville, MD 20852 (US). THE GOVERNMENT OF THE UNITED STATES, represented by THE SECRETARY OF THE ARMY [US/US]; 1600 East Grunde Drive, Rockville, MD 20850 (US).

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(75) Inventors/Applicants *(for US only)*: LOWELL, George, H. [US/US]; 6303 Western Run Drive, Baltimore, MD 21215 (US). VANCOTT, Thomas, C. [US/US]; 19108 Mount Airy Road, Brookesville, MD 20833 (US). BIRX, Deborah, L. [US/US]; 13 Taft Court #200, Rockville, MD 20850 (US).

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(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(88) Date of publication of the international search report:
14 May 1998 (14.05.98)

(54) Title: PROTEIN AND PEPTIDE VACCINES FOR INDUCING MUCOSAL IMMUNITY

(57) Abstract

A novel vaccine composition combines a protein or peptide antigen, optionally added hydrophobic material and an immunopotentiating membranous carrier which together preserve the antigenic integrity of the protein or peptide epitopes while at the same time enhancing their immunogenicity. Administration of this composition to a subject provokes a protective immune response comprising secretory neutralizing antibodies present in various mucosal sites in the body. This vaccine and the process for using it is intended for use against pathogenic organisms, in particular those causing sexually transmitted diseases or mucosally transmitted diseases. Such organisms include bacteria and enveloped viruses, particularly HIV-1.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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EE	Estonia	**LR**	Liberia	**SG**	Singapore		

INTERNATIONAL SEARCH REPORT

Interna[illegible]al Application No
PCT/US 97/12253

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/30 C12N15/31 C12N15/49 A61K39/39 A61K39/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEVI ET AL: "INTRANASAL IMMUNIZATION OF MICE AGAINST INFLUENZA WITH SYNTHETIC PEPTIDES ANCHORED TO PROTEOSOMES" VACCINE, vol. 13, no. 14, 1995, pages 1353-1359, XP002055656	1,3,4,6, 10-20, 25-32
Y	see the whole document and note especially page 1354, paragraph 3 ---	2,5,21
X	WO 95 11700 A (PHARMOS CORP ;US GOVERNMENT (US); LOWELL GEORGE H (US); AMSELEM SH) 4 May 1995	1-4,6-8, 10,11, 13-20, 22,23, 25-32
Y	see page 3, line 32 - page 6, line 15 see page 14, line 13 - page 15, line 9 see page 18 - page 23; examples 1-4 --- -/--	2,5,21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 February 1998	03. 03. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Sitch, W

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LOWELL ET AL: "MUCOSAL IMMUNOGENICITY AND EFFICACY OF PROTEOSOMES AND PA ADJUVANTS FOR HIV, INFLUENZA, SHIGELLA AND STAPH. ENTEROTOXIN B (SEB) VACCINES" JOURNAL OF CELLULAR BIOCHEMISTRY, no. S19A, 1995, page 259 XP002055657 see abstract J1-220 ---	1,2,6-8, 10-12, 16-19, 22,23, 25,26, 30-32
X	KAMINSKI ET AL: "PARENTERAL OR INTRANASAL IMMUNIZATION WITH HIV GP160 FORMULATED WITH PROTEOSOMES AND/OR PA ADJUVANTS ENHANCES EPITOPE-SPECIFIC IGG OR IGA" 94TH GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, 1994, page 155 XP002055658 see abstract E-70 ---	1,2,6-8, 10-12, 16-19, 22,23, 25,26, 30-32
X	LOWELL ET AL: "NASAL IMMUNIZATION WITH HIV GP160 FORMULATED WITH PROTEOSOMES, EMULSOMES AND/OR CHOLERA TOXIN B SUBUNIT: INDUCTION OF ANTI-GP160 SERUM IGA AND IGG AND INTESTINAL, VAGINAL AND LUNG IGA" AMERICAN SOCIETY FOR MICROLBIOLOGY. HUMAN RETROVIRUSES AND RELATED INFECTIONS. 2ND NATIONAL CONFERENCE, - 1995 page 81 XP002055659 see abstract 146 ---	1,2,6-8, 10-12, 16-19, 22,23, 25,26, 30-32
Y	LOWELL ET AL: "PEPTIDES BOUND TO PROTEOSOMES VIA HYDROPHOBIC FEET BECOME HIGHLY IMMUNOGENIC WITHOUT ADJUVANTS" JOURNAL OF EXPERIMENTAL MEDICINE, vol. 167, 1988, pages 658-663, XP002055660 see the whole document ---	2,5,21
P,X	LOWELL ET AL: "PROTEOSOMES, EMULSOMES, AND CHOLERA TOXIN B IMPROVE NASAL IMMUNOGENICITY OF HUMAN IMMUNODEFICIENCY VIRUS GP160 IN MICE: INDUCTION OF SERUM, INTESTINAL, VAGINAL, AND LUNG IGA AND IGG" THE JOURNAL OF INFECTIOUS DISEASES, vol. 175, no. 2, February 1997, pages 292-301, XP002055661 see page 292 see abstract ---	1,2,6-8, 10-12, 16-19, 22,23, 25,26, 30-32

1 -/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KAMINSKI ET AL: "HIV PEPTIDE AND PROTEIN ANTIBODY RESPONSES ELICITED BY IMMUNIZATION WITH RGP 160 FORMULATED WITH PROTEOSOMES, ALUM, AND/OR SUBMICRON EMULSIONS" VACCINE RESEARCH, vol. 4, 1995, pages 189-206, XP002055662 see page 189 see abstract ---	
A	LOWELL ET AL: "PROTEOSOME-LIPOPEPTIDE VACCINES: ENHANCEMENT OF IMMUNOGENICITY FOR MALARIA CS PEPTIDES" SCIENCE, vol. 240, 1988, pages 800-802, XP002055663 see page 800 see abstract ---	
A	FRANKENBURG ET AL: "EFFECTIVE IMMUNIZATION OF MICE AGAINST CUTANEOUS LEISHMANIASIS USING AN INTRINSICALLY ADJUVANTED SYNTHETIC LIPOPEPTIDE VACCINE" VACCINE, vol. 14, no. 9, June 1996, pages 923-929, XP002055664 see page 923 see abstract ---	
A	VANCOTT ET AL: "CHARACTERIZATION OF A SOLUBLE, OLIGOMERIC HIV-1 GP160 PROTEIN AS A POTENTIAL IMMUNOGEN" JOURNAL OF IMMUNOLOGICAL METHODS, vol. 183, 1995, pages 103-117, XP002055665 cited in the application see page 103 see abstract ---	
A	YANG ET AL: "THE HUMAN AND SIMIAN IMMUNODEFICIENCY VIRUS ENVELOPE GLYCOPROTEIN TRANSMEMBRANE SUBUNITS ARE PALMITOYLATED" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, USA, vol. 92, 1995, pages 9871-9875, XP002055666 cited in the application see page 9871 see abstract -----	

INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/US 97/12253

B x I Observations where certain claims were found unsearchabl (Continuati n f item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 19-32 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/12253

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9511700 A	04-05-95	AU 5543294 A	22-05-95

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
359292000240		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 97/12253	10/07/1997	10/07/1996
Applicant		
INTELLIVAX, INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [X] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:
   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/12253

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 19-32 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Interna[redacted] Application No
PCT/US 97/12253

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/30 C12N15/31 C12N15/49 A61K39/39 A61K39/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEVI ET AL: "INTRANASAL IMMUNIZATION OF MICE AGAINST INFLUENZA WITH SYNTHETIC PEPTIDES ANCHORED TO PROTEOSOMES" VACCINE, vol. 13, no. 14, 1995, pages 1353-1359, XP002055656	1,3,4,6, 10-20, 25-32
Y	see the whole document and note especially page 1354, paragraph 3 ---	2,5,21
X	WO 95 11700 A (PHARMOS CORP ;US GOVERNMENT (US); LOWELL GEORGE H (US); AMSELEM SH) 4 May 1995	1-4,6-8, 10,11, 13-20, 22,23, 25-32
Y	see page 3, line 32 - page 6, line 15 see page 14, line 13 - page 15, line 9 see page 18 - page 23; examples 1-4 --- -/--	2,5,21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
13 February 1998	03. 03. 98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Sitch, W

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LOWELL ET AL: "MUCOSAL IMMUNOGENICITY AND EFFICACY OF PROTEOSOMES AND PA ADJUVANTS FOR HIV, INFLUENZA, SHIGELLA AND STAPH. ENTEROTOXIN B (SEB) VACCINES" JOURNAL OF CELLULAR BIOCHEMISTRY, no. S19A, 1995, page 259 XP002055657 see abstract J1-220 ---	1,2,6-8, 10-12, 16-19, 22,23, 25,26, 30-32
X	KAMINSKI ET AL: "PARENTERAL OR INTRANASAL IMMUNIZATION WITH HIV GP160 FORMULATED WITH PROTEOSOMES AND/OR PA ADJUVANTS ENHANCES EPITOPE-SPECIFIC IGG OR IGA" 94TH GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, 1994, page 155 XP002055658 see abstract E-70 ---	1,2,6-8, 10-12, 16-19, 22,23, 25,26, 30-32
X	LOWELL ET AL: "NASAL IMMUNIZATION WITH HIV GP160 FORMULATED WITH PROTEOSOMES, EMULSOMES AND/OR CHOLERA TOXIN B SUBUNIT: INDUCTION OF ANTI-GP160 SERUM IGA AND IGG AND INTESTINAL, VAGINAL AND LUNG IGA" AMERICAN SOCIETY FOR MICROLBIOLOGY. HUMAN RETROVIRUSES AND RELATED INFECTIONS. 2ND NATIONAL CONFERENCE, - 1995 page 81 XP002055659 see abstract 146 ---	1,2,6-8, 10-12, 16-19, 22,23, 25,26, 30-32
Y	LOWELL ET AL: "PEPTIDES BOUND TO PROTEOSOMES VIA HYDROPHOBIC FEET BECOME HIGHLY IMMUNOGENIC WITHOUT ADJUVANTS" JOURNAL OF EXPERIMENTAL MEDICINE, vol. 167, 1988, pages 658-663, XP002055660 see the whole document ---	2,5,21
P,X	LOWELL ET AL: "PROTEOSOMES, EMULSOMES, AND CHOLERA TOXIN B IMPROVE NASAL IMMUNOGENICITY OF HUMAN IMMUNODEFICIENCY VIRUS GP160 IN MICE: INDUCTION OF SERUM, INTESTINAL, VAGINAL, AND LUNG IGA AND IGG" THE JOURNAL OF INFECTIOUS DISEASES, vol. 175, no. 2, February 1997, pages 292-301, XP002055661 see page 292 see abstract ---	1,2,6-8, 10-12, 16-19, 22,23, 25,26, 30-32

1 -/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KAMINSKI ET AL: "HIV PEPTIDE AND PROTEIN ANTIBODY RESPONSES ELICITED BY IMMUNIZATION WITH RGP 160 FORMULATED WITH PROTEOSOMES, ALUM, AND/OR SUBMICRON EMULSIONS" VACCINE RESEARCH, vol. 4, 1995, pages 189-206, XP002055662 see page 189 see abstract ---	
A	LOWELL ET AL: "PROTEOSOME-LIPOPEPTIDE VACCINES: ENHANCEMENT OF IMMUNOGENICITY FOR MALARIA CS PEPTIDES" SCIENCE, vol. 240, 1988, pages 800-802, XP002055663 see page 800 see abstract ---	
A	FRANKENBURG ET AL: "EFFECTIVE IMMUNIZATION OF MICE AGAINST CUTANEOUS LEISHMANIASIS USING AN INTRINSICALLY ADJUVANTED SYNTHETIC LIPOPEPTIDE VACCINE" VACCINE, vol. 14, no. 9, June 1996, pages 923-929, XP002055664 see page 923 see abstract ---	
A	VANCOTT ET AL: "CHARACTERIZATION OF A SOLUBLE, OLIGOMERIC HIV-1 GP160 PROTEIN AS A POTENTIAL IMMUNOGEN" JOURNAL OF IMMUNOLOGICAL METHODS, vol. 183, 1995, pages 103-117, XP002055665 cited in the application see page 103 see abstract ---	
A	YANG ET AL: "THE HUMAN AND SIMIAN IMMUNODEFICIENCY VIRUS ENVELOPE GLYCOPROTEIN TRANSMEMBRANE SUBUNITS ARE PALMITOYLATED" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, USA, vol. 92, 1995, pages 9871-9875, XP002055666 cited in the application see page 9871 see abstract -----	

1

INTERNATIONAL SEARCH REPORT

Informa[redacted] patent family members

Interna[redacted] Application No
PCT/[redacted] 97/12253

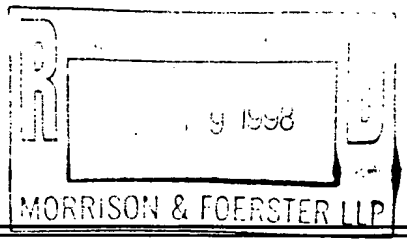
Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9511700 A	04-05-95	AU 5543294 A	22-05-95

gdc/AB
10.19.98

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: THOMAS G. WISEMAN
MORRISON & FOERSTER LLP
2000 PENNSYLVANIA AVENUE, N.W.
WASHINGTON DC 20006



PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	16 OCT 1998
Applicant's or agent's file reference 359292000240	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US97/12253	10 JULY 1997	10 JULY 1996

Applicant
INTELLIVAX, INC.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

DOCKETED

Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer Jeffrey S. Parkin, Ph.D.
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/416 (July 1992)★

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 359292000240	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US97/12253	International filing date *(day/month/year)* 10 JULY 1997	Priority date *(day/month/year)* 10 JULY 1996
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant INTELLIVAX, INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of report with regard to novelty, inventive step or industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
15 JANUARY 1998	17 SEPTEMBER 1998
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Jeffrey S. Pakrin, Ph.D. Telephone No. (703) 308-0196

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US97/12253

I. Basis of the report

1. This report has been drawn on the basis of *(Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments).*

[X] the international application as originally filed.

[X] the description, pages 1-52 , as originally filed.
pages NONE , filed with the demand.
pages NONE , filed with the letter of _____.
pages _____ , filed with the letter of _____.

[X] the claims, Nos. 1-32 , as originally filed.
Nos. NONE , as amended under Article 19.
Nos. NONE , filed with the demand.
Nos. NONE , filed with the letter of _____.
Nos. _____ , filed with the letter of _____.

[X] the drawings, sheets/~~fig~~ 1-4 , as originally filed.
sheets/~~fig~~ NONE , filed with the demand.
sheets/~~fig~~ NONE , filed with the letter of _____.
sheets/~~fig~~ _____ , filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

[X] the description, pages NONE.

[X] the claims, Nos. NONE.

[X] the drawings, sheets/~~fig~~ NONE.

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the ~~Supplemental Box~~ Additional observations below (Rule 70.2(c)).

4. Additional observations, if necessary:

NONE

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US97/12253

III. Non-establishment f opinion with regard to novelty, inventive step and industrial applicability

The question wh ther the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application.

☒ claims Nos. 9 and 24

because:

☐ the said international application, or the said claim Nos. _ relate to the following subject matter which does not require international preliminary examination *(specify)*.

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 9 and 24 are so unclear that no meaningful opinion could be formed *(specify)*.

Applicants have failed to provide a sequence listing in electronic format (e.g., machine readable form) for claims 9 and 24. Pursuant to PCT Rule 13ter.1, these claims have not been examined.

☐ the claims, or said claims Nos. _ are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos. _.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US97/12253

V. Reas ned statement under Articl 35(2) with regard to novelty, inventiv step r industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO
Inventive Step (IS)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO
Industrial Applicability (IA)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO

2. CITATIONS AND EXPLANATIONS

Claims 1-7, 11-14, 17, 19-22, 25, 27, and 28 lack novelty under PCT Article 33(2) as being anticipated by Lowell *et al.* (1988, J. Exp. Med., 167:658-663), hereafter referred to as Lowell *et al.* (1988a). Lowell *et al.* (1988a) teach the preparation of vaccine compositions comprising an antigen with endogenous hydrophobic sequence of between about 3 and about 50 amino acids coupled to an exogenous hydrophobic sequence, which in turn is complexed with a proteosomes to form a proteosomal composition (refer to page 659, first paragraph and Materials and Methods). This teaching also discloses the coupling of lauroyl or pentapeptide (Phe-Leu-Leu-Ala-Val) groups to the antigen of interest to facilitate proteosomal complex formation. Proteosomal complexes were formed in the presence of detergent which was subsequently removed through dialysis. This teaching meets all the limitations of the claimed invention.

Claims 1-7, 11-14, 17, 19-22, 25, 27, and 28 lack novelty under PCT Article 33(2) as being anticipated by Lowell *et al.* (1988, Science, 240:800-802), hereafter referred to as Lowell *et al.* (1988b). Lowell *et al.* (1988b) teach the preparation of vaccine compositions comprising an antigen with endogenous hydrophobic sequence of between about 3 and about 50 amino acids coupled to an exogenous hydrophobic sequence, which in turn is complexed with a proteosomes to form a proteosomal composition (refer to page 659, first paragraph and Materials and Methods). This teaching also discloses the coupling of lauroyl or pentapeptide (Phe-Leu-Leu-Ala-Val) groups to the antigen of interest to facilitate proteosomal complex formation. Proteosomal complexes were formed in the presence of detergent which was subsequently removed through dialysis. Cysteine residues were also added between the antigen and hydrophobic foot to enhance the immunogenic properties of the vaccine composition. This teaching meets all the limitations of the claimed invention.

Claims 8, 10, 15, 16, 18, 23, 26, and 29-32 lack an inventive step under PCT Article 33(3) as being obvious over Lowell *et al.* (1988a, 1988b) in view of VanCott *et al.* (1995) and Levi *et al.* (1995). Lowell *et al.* (1988a) disclose the preparation of (Continued on Supplemental Sheet.)

Supplemental B x
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(6): A61K 38/00; C07K 1/00; A61K 39/21, 39/385, 45/00 and US Cl.: 530/300, 402, 403; 424/188.1, 193.1, 278.1, 283.1

V. 1. REASONED STATEMENTS:
The report as to Novelty was positive (YES) with respect to claims 8, 10, 15, 16, 18, 23, 26, and 29-32.
The report as to Novelty was negative (NO) with respect to claims 1-7, 11-14, 17, 19-22, 25, 27, and 28.
The report as to Inventive Step was positive (YES) with respect to claims NONE.
The report as to Inventive Step was negative (NO) with respect to claims 1-8, 10-23, and 25-32.
The report as to Industrial Applicability was positive (YES) with respect to claims 1-8, 10-23, and 25-32.
The report as to Industrial Applicability was negative (NO) with respect to claims NONE.

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
vaccine compositions comprising an antigen with endogenous hydrophobic sequence of between about 3 and about 50 amino acids coupled to an exogenous hydrophobic sequence, which in turn is complexed with a proteosomes to form a proteosomal composition (refer to page 659, first paragraph and Materials and Methods). This teaching also discloses the coupling of lauroyl or pentapeptide (Phe-Leu-Leu-Ala-Val) groups to the antigen of interest to facilitate proteosomal complex formation. Proteosomal complexes were formed in the presence of detergent which was subsequently removed through dialysis. This teaching does not specifically describe vaccine compositions comprising HIV gp160 antigens or the administration of said vaccine compositions via intranasal or respiratory routes.

Lowell *et al.* (1988b) teaches the preparation of vaccine compositions comprising an antigen with endogenous hydrophobic sequence of between about 3 and about 50 amino acids coupled to an exogenous hydrophobic sequence, which in turn is complexed with a proteosomes to form a proteosomal composition (refer to page 659, first paragraph and Materials and Methods). This teaching also discloses the coupling of lauroyl or pentapeptide (Phe-Leu-Leu-Ala-Val) groups to the antigen of interest to facilitate proteosomal complex formation. Proteosomal complexes were formed in the presence of detergent which was subsequently removed through dialysis. Cysteine residues were also added between the antigen and hydrophobic foot to enhance the immunogenic properties of the vaccine composition. This teaching also fails to disclose vaccine compositions comprising HIV gp160 antigens or the administration of said vaccine compositions via intranasal or respiratory routes.

VanCott *et al.* (1995) teaches that oligomeric HIV gp160 displays high reactivity toward divergent mAbs and should be included in potential HIV vaccines (see page 103, Abstract and page 115, Discussion). Levi *et al.* (1995) teaches that the intranasal immunization of mammals with proteosomal vaccines confers protection following viral challenge. Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to utilize known immunogens derived from infectious agents, as taught by VanCott *et al.* (1995) and Levi *et al.* (1995), in the proteosomal compositions described by Lowell *et al.* (1988a, 1988b), since this represents an efficient means for generating antigen-specific immune responses. One of ordinary skill in the art would be motivated to utilize different immunization sites (e.g., intranasal) and regimens depending upon the nature of the immune response desired (e.g., mucosal). Finally, one of ordinary skill in the art could employ lyophilization, or other art-recognized methods of vaccine preparation, to make the proteosomal compositions.

———— NEW CITATIONS ————

LOWELL et al. Peptides Bound to Proteosomes via Hydrophobic Feet Become Highly Immunogenic Without Adjuvants. J. Exp. Med. February 1988, Vol. 167, pages 658-663, see entire document.

LOWELL et al. Proteosome-Lipopeptide Vaccines: Enhancement of Immunogenicity for Malaria CS Peptides. Science. 06 May 1988, Vol. 240, pages 800-802, see entire document.

VANCOTT et al. Characterization of a Soluble, Oligomeric HIV-1 gp160 Protein as a Potential Immunogen. J. Immunol. Methods. 1995, Vol. 183, pages 103-117, see entire document.

LEVI et al. Intranasal Immunization of Mice Against Influenza with Synthetic Peptides Anchored to Proteosomes. Vaccine. 1995, Vol. 13, No. 14, pages 1353-1359, see entire document.

rdc/ABM 3.13.98

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
MORRISON & FOERSTER
Attn. WISEMAN, Thomas G.
2000 Pennsylvania Avenue, N.W.
Washington, D.C. 20006-1812
UNITED STATES OF AMERICA

Date of mailing *(day/month/year)* 03/03/1998	
Applicant's or agent's file reference 359292000240	**FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/US 97/12253	International filing date *(day/month/year)* 10/07/1997
Applicant INTELLIVAX, INC. et al.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

DOCKETED
RS Due 4.3.98
LD 5.3.98

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicants's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Monika Schmitz MAR 9 1998

Form PCT/ISA/220 (January 1994)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if th language of the international application is French, the letter must be in French.

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The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
359292000240		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 97/12253	10/07/1997	10/07/1996
Applicant		
INTELLIVAX, INC. et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [X] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [X] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [X] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 97/12253

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 19-32 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/12253

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/30 C12N15/31 C12N15/49 A61K39/39 A61K39/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LEVI ET AL: "INTRANASAL IMMUNIZATION OF MICE AGAINST INFLUENZA WITH SYNTHETIC PEPTIDES ANCHORED TO PROTEOSOMES" VACCINE, vol. 13, no. 14, 1995, pages 1353-1359, XP002055656	1,3,4,6, 10-20, 25-32
Y	see the whole document and note especially page 1354, paragraph 3 ---	2,5,21
X	WO 95 11700 A (PHARMOS CORP ;US GOVERNMENT (US); LOWELL GEORGE H (US); AMSELEM SH) 4 May 1995	1-4,6-8, 10,11, 13-20, 22,23, 25-32
Y	see page 3, line 32 - page 6, line 15 see page 14, line 13 - page 15, line 9 see page 18 - page 23; examples 1-4 --- -/--	2,5,21

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
13 February 1998	03.03.98
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	**Authorized officer** Sitch, W

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LOWELL ET AL: "MUCOSAL IMMUNOGENICITY AND EFFICACY OF PROTEOSOMES AND PA ADJUVANTS FOR HIV, INFLUENZA, SHIGELLA AND STAPH. ENTEROTOXIN B (SEB) VACCINES" JOURNAL OF CELLULAR BIOCHEMISTRY, no. S19A, 1995, page 259 XP002055657 see abstract J1-220 ---	1,2,6-8, 10-12, 16-19, 22,23, 25,26, 30-32
X	KAMINSKI ET AL: "PARENTERAL OR INTRANASAL IMMUNIZATION WITH HIV GP160 FORMULATED WITH PROTEOSOMES AND/OR PA ADJUVANTS ENHANCES EPITOPE-SPECIFIC IGG OR IGA" 94TH GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, 1994, page 155 XP002055658 see abstract E-70 ---	1,2,6-8, 10-12, 16-19, 22,23, 25,26, 30-32
X	LOWELL ET AL: "NASAL IMMUNIZATION WITH HIV GP160 FORMULATED WITH PROTEOSOMES, EMULSOMES AND/OR CHOLERA TOXIN B SUBUNIT: INDUCTION OF ANTI-GP160 SERUM IGA AND IGG AND INTESTINAL, VAGINAL AND LUNG IGA" AMERICAN SOCIETY FOR MICROLBIOLOGY. HUMAN RETROVIRUSES AND RELATED INFECTIONS. 2ND NATIONAL CONFERENCE, - 1995 page 81 XP002055659 see abstract 146 ---	1,2,6-8, 10-12, 16-19, 22,23, 25,26, 30-32
Y	LOWELL ET AL: "PEPTIDES BOUND TO PROTEOSOMES VIA HYDROPHOBIC FEET BECOME HIGHLY IMMUNOGENIC WITHOUT ADJUVANTS" JOURNAL OF EXPERIMENTAL MEDICINE, vol. 167, 1988, pages 658-663, XP002055660 see the whole document ---	2,5,21
P,X	LOWELL ET AL: "PROTEOSOMES, EMULSOMES, AND CHOLERA TOXIN B IMPROVE NASAL IMMUNOGENICITY OF HUMAN IMMUNODEFICIENCY VIRUS GP160 IN MICE: INDUCTION OF SERUM, INTESTINAL, VAGINAL, AND LUNG IGA AND IGG" THE JOURNAL OF INFECTIOUS DISEASES, vol. 175, no. 2, February 1997, pages 292-301, XP002055661 see page 292 see abstract ---	1,2,6-8, 10-12, 16-19, 22,23, 25,26, 30-32

1 -/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KAMINSKI ET AL: "HIV PEPTIDE AND PROTEIN ANTIBODY RESPONSES ELICITED BY IMMUNIZATION WITH RGP 160 FORMULATED WITH PROTEOSOMES, ALUM, AND/OR SUBMICRON EMULSIONS" VACCINE RESEARCH, vol. 4, 1995, pages 189-206, XP002055662 see page 189 see abstract ---	
A	LOWELL ET AL: "PROTEOSOME-LIPOPEPTIDE VACCINES: ENHANCEMENT OF IMMUNOGENICITY FOR MALARIA CS PEPTIDES" SCIENCE, vol. 240, 1988, pages 800-802, XP002055663 see page 800 see abstract ---	
A	FRANKENBURG ET AL: "EFFECTIVE IMMUNIZATION OF MICE AGAINST CUTANEOUS LEISHMANIASIS USING AN INTRINSICALLY ADJUVANTED SYNTHETIC LIPOPEPTIDE VACCINE" VACCINE, vol. 14, no. 9, June 1996, pages 923-929, XP002055664 see page 923 see abstract ---	
A	VANCOTT ET AL: "CHARACTERIZATION OF A SOLUBLE, OLIGOMERIC HIV-1 GP160 PROTEIN AS A POTENTIAL IMMUNOGEN" JOURNAL OF IMMUNOLOGICAL METHODS, vol. 183, 1995, pages 103-117, XP002055665 cited in the application see page 103 see abstract ---	
A	YANG ET AL: "THE HUMAN AND SIMIAN IMMUNODEFICIENCY VIRUS ENVELOPE GLYCOPROTEIN TRANSMEMBRANE SUBUNITS ARE PALMITOYLATED" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, USA, vol. 92, 1995, pages 9871-9875, XP002055666 cited in the application see page 9871 see abstract -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 97/12253

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9511700 A	04-05-95	AU 5543294 A	22-05-95

Supplemental B x
(T be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(6): A61K 38/00; C07K 1/00; A61K 39/21, 39/385, 45/00 and US Cl.: 530/300, 402, 403; 424/188.1, 193.1, 278.1, 283.1

V. 1. REASONED STATEMENTS:
The report as to Novelty was positive (YES) with respect to claims 8, 10, 15, 16, 18, 23, 26, and 29-32.
The report as to Novelty was negative (NO) with respect to claims 1-7, 11-14, 17, 19-22, 25, 27, and 28.
The report as to Inventive Step was positive (YES) with respect to claims NONE.
The report as to Inventive Step was negative (NO) with respect to claims 1-8, 10-23, and 25-32.
The report as to Industrial Applicability was positive (YES) with respect to claims 1-8, 10-23, and 25-32.
The report as to Industrial Applicability was negative (NO) with respect to claims NONE.

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
vaccine compositions comprising an antigen with endogenous hydrophobic sequence of between about 3 and about 50 amino acids coupled to an exogenous hydrophobic sequence, which in turn is complexed with a proteosomes to form a proteosomal composition (refer to page 659, first paragraph and Materials and Methods). This teaching also discloses the coupling of lauroyl or pentapeptide (Phe-Leu-Leu-Ala-Val) groups to the antigen of interest to facilitate proteosomal complex formation. Proteosomal complexes were formed in the presence of detergent which was subsequently removed through dialysis. This teaching does not specifically describe vaccine compositions comprising HIV gp160 antigens or the administration of said vaccine compositions via intranasal or respiratory routes.

Lowell *et al.* (1988b) teaches the preparation of vaccine compositions comprising an antigen with endogenous hydrophobic sequence of between about 3 and about 50 amino acids coupled to an exogenous hydrophobic sequence, which in turn is complexed with a proteosomes to form a proteosomal composition (refer to page 659, first paragraph and Materials and Methods). This teaching also discloses the coupling of lauroyl or pentapeptide (Phe-Leu-Leu-Ala-Val) groups to the antigen of interest to facilitate proteosomal complex formation. Proteosomal complexes were formed in the presence of detergent which was subsequently removed through dialysis. Cysteine residues were also added between the antigen and hydrophobic foot to enhance the immunogenic properties of the vaccine composition. This teaching also fails to disclose vaccine compositions comprising HIV gp160 antigens or the administration of said vaccine compositions via intranasal or respiratory routes.

VanCott *et al.* (1995) teaches that oligomeric HIV gp160 displays high reactivity toward divergent mAbs and should be included in potential HIV vaccines (see page 103, Abstract and page 115, Discussion). Levi *et al.* (1995) teaches that the intranasal immunization of mammals with proteosomal vaccines confers protection following viral challenge. Therefore, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to utilize known immunogens derived from infectious agents, as taught by VanCott *et al.* (1995) and Levi *et al.* (1995), in the proteosomal compositions described by Lowell *et al.* (1988a, 1988b), since this represents an efficient means for generating antigen-specific immune responses. One of ordinary skill in the art would be motivated to utilize different immunization sites (e.g., intranasal) and regimens depending upon the nature of the immune response desired (e.g., mucosal). Finally, one of ordinary skill in the art could employ lyophilization, or other art-recognized methods of vaccine preparation, to make the proteosomal compositions.

———— NEW CITATIONS ————

LOWELL et al. Peptides Bound to Proteosomes via Hydrophobic Feet Become Highly Immunogenic Without Adjuvants. J. Exp. Med. February 1988, Vol. 167, pages 658-663, see entire document.

LOWELL et al. Proteosome-Lipopeptide Vaccines: Enhancement of Immunogenicity for Malaria CS Peptides. Science. 06 May 1988, Vol. 240, pages 800-802, see entire document.

VANCOTT et al. Characterization of a Soluble, Oligomeric HIV-1 gp160 Protein as a Potential Immunogen. J. Immunol. Methods. 1995, Vol. 183, pages 103-117, see entire document.

LEVI et al. Intranasal Immunization of Mice Against Influenza with Synthetic Peptides Anchored to Proteosomes. Vaccine. 1995, Vol. 13, No. 14, pages 1353-1359, see entire document.

For receiving Office use only

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

International Application No.
International Filing Date
Name of receiving Office and "PCT International Application"
Applicant's or agent's file reference *359292000240*

Box No. I TITLE OF INVENTION

PROTEIN AND PEPTIDE VACCINES FOR INDUCING MUCOSAL IMMUNITY

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*

☐ This person is also inventor.

INTELLIVAX, INC.
6303 Western Run Drive
Baltimore, MD 21215
US

Telephone No.:

Facsimile No.:

Teleprinter No.

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of:

☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

Name and address:

HENRY M. JACKSON FOUNDATION
1401 Rockville Pike, Suite 600
Rockville, MD 20852
US

This person is:
☒ applicant only
☐ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: US

State *(i.e. country)* of residence: US

This person is applicant for the purposes of:

☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE: OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.*

WISEMAN, Thomas G.
Morrison & Foerster LLP
2000 Pennsylvania Avenue, N.W.
Washington, D.C. 20006-1888
USA

Telephone No.
(202) 887-1678

Facsimile No.
(202) 887-0763

Teleprinter No.

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

See Notes to the request form

Continuation of Box No. III **FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS**

If none of the following sub-boxes is used, this sheet is not to be included in the request

Name and address:

GOVERNMENT OF THE UNITED STATES AS REPRESENTED BY THE SECRETARY OF THE ARMY
1600 East Grunde Drive
Rockville, MD 20850
US

This person is:
☒ applicant only
☐ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State (*i.e. country*) of nationality: US

State (*i.e. country*) of residence: US

This person is applicant for the purposes of:
☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address:

LOWELL, George H.
6303 Western Run Drive
Baltimore, MD 21215
US

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State (*i.e. country*) of nationality: US

State (*i.e. country*) of residence: US

This person is applicant for the purposes of:
☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address:

VANCOTT, Thomas C.
19108 Mount Airy Road
Brookesville, MD 20833
US

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State (*i.e. country*) of nationality: US

State (*i.e. country*) of residence: US

This person is applicant for the purposes of:
☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address:

BIRX, Deborah L.
13 Taft Court, #200
Rockville, MD 20850
US

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State (*i.e. country*) of nationality: US

State (*i.e. country*) of residence: US

This person is applicant for the purposes of:
☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No. V DESIGNATION OF STA[illegible]

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- ☒ AP **ARIPO Patent:** KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, and any other State which is a Contracting State of the Harare Protocol and of the PCT.
- ☒ EA **Eurasian Patent:** AZ Azerbaijan, BY Belarus, KZ Kazakstan, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT.
- ☒ EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany, DK Denmark, ES Spain, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT.
- ☒ OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒	AL	Albania
☒	AM	Armenia
☒	AT	Austria
☒	AU	Australia
☒	AZ	Azerbaijan
☒	BB	Barbados
☒	BG	Bulgaria
☒	BR	Brazil
☒	BY	Belarus
☒	CA	Canada
☒	CH and LI	Switzerland and Liechtenstein
☒	CN	China
☒	CU	Cuba
☒	CZ	Czech Republic
☒	DE	Germany
☒	DK	Denmark
☒	EE	Estonia
☒	ES	Spain
☒	FI	Finland
☒	GB	United Kingdom
☒	GE	Georgia
☒	HU	Hungary
☒	IL	Isarel
☒	IS	Iceland
☒	JP	Japan
☒	KE	Kenya
☒	KG	Kyrgyzstan
☒	KP	Democratic People's Republic of Korea
☒	KR	Republic of Korea
☒	KZ	Kazakhstan
☒	LC	St. Lucia
☒	LK	Sri Lanka
☒	LR	Liberia
☒	LS	Lesotho
☒	LT	Lithuania
☒	LU	Luxembourg
☒	LV	Latvia
☒	MD	Republic of Moldova
☒	MG	Madagascar
☒	MK	The former Yugoslav Republic of Macedonia
☒	MN	Mongolia
☒	MW	Malawi
☒	MX	Mexico
☒	NO	Norway
☒	NZ	New Zealand
☒	PL	Poland
☒	PT	Portugal
☒	RO	Romania
☒	RU	Russian Federation
☒	SD	Sudan
☒	SE	Sweden
☒	SG	Singapore
☒	SI	Slovenia
☒	SK	Slovakia
☒	TJ	Tajikistan
☒	TM	Turkmenistan
☒	TR	Turkey
☒	TT	Trinidad and Tobago
☒	UA	Ukraine
☒	UG	Uganda
☒	US	United States of America CIP of USSN 60/021,687 filed 10 July 1996
☒	UZ	Uzbekistan
☒	VN	Viet Nam

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☐ .....
- ☐ .....
- ☐ .....
- ☐ .....

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____. **The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that timellimit.** *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Supplemental Box *If the Supplemental Box is not used, this sheet need not be included in the request.*	
Use this box in the following cases: *1. If, in any of the Boxes, the space is insufficient to furnish all the information:* *in particular:*	*in such case write "Continuation of Box No. ..." (indicate the number of the Box) and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient;*
(i) if more than three persons are involved as applicants and/or inventors and no "continuation sheet" is available;	*in such case, write "continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III;*
(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked;	*in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and III" (as the case may be), indicate the name of the applicant(s) and, next to (each) such name, the State or States (and/or, where applicable, European or OAPI patent) for the purposes of which the named person is applicant;*
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(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents;	*in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*
(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition", "certificate of addition" or "inventor's certificate of addition", or if, in Box No. V, the name of the United States of America is accompanied by an indication "Continuation" or "Continuation-in-part";	*in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*
(vi) if there are more than three earlier applications whose priority is claimed.	*in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.*
2. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty:	*in such case, write "Statement Concerning Non-Prejudicial Disclosures or Exceptions to Lack of Novelty" and furnish that statement below.*

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BOX No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country *(in which, or for which, the application was filed)*	Filing Date *(day/month/year)*	Application No.	Office of filing *(only for regional or international application)*
item (1) US	10 July 1996 [10.07.96]	60/021.687	
item (2)			
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☒ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s): (1)

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) (If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used: ISA/EP

Earlier search Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:

Country *(or regional Office)*: * Date *(day/month/year)*: * Number: *

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	:	5	sheets
2. description	:	52	sheets
3. claims	:	5	heets
4. abstract	:	1	sheets
5. drawings	:	4	sheets
Total	:	67	sheets

This international application contains the following number of sheets:

1. ☐ Separate signed power of attorney
2. ☐ Copy of General Power of Attorney
3. ☐ Statement Explaining lack of signature
4. ☐ Priority Document(s)
5. ☒ Fee Calculation Sheet
6. ☐ Separate indications concerning deposited microorganisms
7. ☐ Nucleotide and/or amino acid sequence listing (diskette)
8. ☒ Other: Transmittal Letter Postcard

Figure No. ___ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Shmuel Livnat

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1. Date of actual receipt of the purported international application:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority specified by the applicant: ISA/
6. ☐ Transmittal of search copy delayed until search fee is paid.

2. Drawings:
   ☐ received:
   ☐ not received:

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Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

PCT
FEE CALCULATION SHEET
Annex to the Request

For receiving Office use only
International application No.

Applicant's or agent's file reference 359292000240

Date stamping of the receiving Office

Applicant *INTELLIVAX et al.*

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE	230.00	[T]	
2. SEARCH FEE	1,585.00	[S]	

International search to be carried out by EP
(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee
The international application contains 67 sheets.

first 30 sheets	590.00	[b_1]	
37 remaining sheets x 12.00 additional amount =	440.00	[b_2]	
Add amounts entered of b_1 and b_2 and enter total at B	1,034.00	[B]	

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11 number of designations x 143.00 amount of designation fee =	1,573.00	[D]	

(If that total exceeds the figure which corresponds to the amount of the designation fee multiplied by ten, enter the latter figure in box D.)

Add amounts entered at B and D and enter total at I *(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the total to be entered at I is 25% of the sum of the amounts entered at B and D.)*	2,607.00	[I]	
4. FEE FOR PRIORITY DOCUMENT	15.00	[P]	
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☒ authorization to charge deposit account (see below)
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☒ is hereby authorized to charge the total fees indicated above to my deposit account.
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03-1952
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TABLE 2

Anti-Peptide Antibody Titers In Sera of Mice After Primary (1°), Secondary (2°) and Tertiary (3°) Immunization with Peptides with Lauroyl Or FLLAV Hydrophobic Feet and/or Cysteine and/or Proteosomes

MOUSE NO.	STRAIN§	IMMUNIZATION WITH:	SERUM ANTIBODY ANTI-PEPTIDE TITERS POST VACCINE		
			1°	2°	3°
1	B, J	pepG Controls (a-e)*	<50	<50	<50
2	B	Proteosome-Lauroyl-pepG	400	204,800	204,800
3	B	Proteosome-FLLAV-pepG	400	12,800	102,400
4	J	Proteosome-Lauroyl-pepG	200	6,400	51,200
5	J	Proteosome-FLLAV-pepG	100	102,400	409,600
6	B	pepM1 Controls (a-c)*	<50	<50	<50
7	B	Lauroyl-pepM1	<50	200	400
8	B	Proteosome-Lauroyl-pepM1	<50	400	400
9	B	pepCM1 Controls (a-c)*	<50	<50	<50
10	B	Lauroyl-pepCM1	<50	<50	3,200
11	B	Proteosome-Lauroyl-pepCM1	400	102,400	409,600
12	B	Proteosome-Lauroyl-pepCM1 (8ug)	200	102,400	204,900
13	B, J	pepCL1 Controls (a-d)*	<50	<50	<50
14	B	Lauroyl-pepCL1	800	400	800
15	B	Proteosome-Lauroyl-pepCL1	50	200	51,200
16	J	Proteosome-Lauroyl-pepCL1	50	400	51,200

§ Groups of 5-8 BALB/c (B) or C3H/HeJ (J) mice were immunized ip on weeks 0, 3 and 7 with vaccines containing 40 μg of peptide; sera, obtained 2-3 weeks after each immunization, were tested in an ELISA for IgG antibodies against the homologous peptide (either pepG, pepM1 or pepL1). Titers are the highest serum dilutions which had ELISA values that were a) more than 0.1 OD units and b) twice the value of pre-vaccination sera diluted 1:50. *Each of the Control groups consisted of 5 mice immunized with either a) peptide alone, b) peptide in Freund's adjuvant, c) peptide and Proteosomes without hydrophobic feet, d) Lauroyl peptide without proteosomes, and e) FLLAV-peptide without Proteosomes.

TABLE 3

Anti-Peptide Antibody Titers in Sera of Mice After Primary (1°), Secondary (2°) and Tertiary (3°) Immunizations with Peptides with Lauroyl or FLLAV Hydrophobic Feet and/or Cysteines and/or Replicated Epitopes and/or Proteosomes

GRP	MOUSE STRAIN	VACCINE	ANTI-PEPTIDE SERUM ANTIBODY TITERS POST-IMMUNIZATION		
			1°	2°	3°
17	B, J	pepCM3 Control groups (a-c)*	<50	<50	<50
18	B	Lauroyl-pepCM4	400	102,400	102,400
19	B	Lauroyl-pepcM3 (non-dialyzed)	<50	<50	100
20	B	Proteosome-Lauroyl-pepCM3	6,400	102,800	409,600
21	B	FLLAV-pepCM3	<50	50	50
22	B	Proteosome-FLLAV-pepCM3	<50	204,800	6,553,600
23	J	Lauroyl-pepCM3	<50	50	50
24	J	Proteosome-auroyl-pepCM3	<50	800	204,800
25	B	pepM5 Control groups (a-c)*	<50	<50	<50
26	B	Lauroyl-pepM5	200	400	12,800
27	B	Proteosome-Lauroyl-pepM5	200	1600	12,800
28	B, J	pepCM5 Control groups (a-c)*	<50	<50	<50
29	B	Lauroyl-pepCM5	800	204,800	204,800
30	B	Lauroyl-pepCM5 (non-dialyzed)	100	12,800	25,600
31	B	Proteosome-Lauroyl-pepCM5	400	15,600	3,276,800
32	J	Lauroyl-pepCM5	50	100	100
33	J	Proteosome-Lauroyl-pepCM5	200	25,600	51,200

§ Groups of 5-8 BALB/c (B) or C3H/HeJ (J) mice were immunized ip with vaccines containing 40 μg of peptide on weeks 0, 3 & 7; sera, obtained 2-3 weeks after each immunization, were tested in an ELISA for anti-pepMl IgG. Titers shown are the highest serum dilutions with ELISA values that were both a) >0.1 O.D. units and b) twice the value of pre-vaccination sera diluted 1:50.

* Each of the Control groups consisted of 5 mice immunized with either (a) peptide alone, (b) peptide in Freund's adjuvant, (c) peptide and proteosomes without hydrophobic feet, (d) lauroyl peptide without proteosomes, and (e) FLLAV-peptide without proteosomes.

*The detergent (Empigen) was removed from the proteosomes by ethanol precipitation and the proteosomes were washed and resuspended in saline prior to mixing (group 34) or lyophilization (group 35) with a saline solution of pepCM1.

§ Groups of 5-8 C57B1/6 mice were immunized ip on weeks 0, 3 and 7 with 40 μg of peptide and the corresponding amount of proteosomes obtained 2-3 weeks after each immunization, were tested in an ELISA for IgG antibodies directed against the homologous peptide, pepMl; titers shown are the highest serum dilutions that had ELISA values that were both a) greater than 0.1 O.D. units and b) twice the value of pre-vaccination sera diluted 1:50.

TABLE 4

Effects of the Complexing Method and the Proteosome:Peptide Ratio on the Ability of Proteosomes to Enhance the Immunogenicity of Peptide Lauroyl-Cm1

GRP No.	Method of Complexing	Proteosome: Peptide RATIO	ANTI-PEPTIDE SERUM ANTIBODY TITERS POST IMMUNIZATION§		
			1°	2°	3°
35	Lyophilize	1:1	800	12,800	409,600
34	Mix	1:1	400	6,400	51,200
36	Dialyze	1:1	12,800	409,600	6,553,600
37	Dialyze	1:2	25,600	819,200	819,000
38	Dialyze	1:4	6,400	819,200	1,638,400
39	Dialyze	1:8	12,800	819,200	1,638,400
40	Dialyze	1:16	51,200	1,638,400	3,276,800

§ Groups of 5-8 BALB/c or C3H/HeJ mice were immunized ip on weeks 0, 3 and 7 with vaccines containing 40 μg of peptide; sera, obtained 2-3 weeks after each immunization, were tested in an ELISA for IgG antibodies against meningococcal outer membrane proteins. Titers shown are the highest serum dilutions obtained after two or three immunizations which had ELISA values that were (a) more than 0.1 OD. units and (b) twice the value of pre-vaccination sera diluted 1:50.

TABLE 5

Anti-Meningococcal IgG Antibodies in Sera of Mice Immunized and Boosted with Proteosome-Hydrophobic Foot-Peptide Vaccines Using Either Lauroyl or FLLAV Hydrophobic Foot

GRP NO.	Immunization	Anti-Meningococcal IgG Vaccine Antibody Titers §
1	Controls	<50
2	Proteosome-Lauroyl-pepG	102,400
4	Proteosome-FLLAV-pepG	409,600

§ Groups of 5-8 BALB/c or C3H/HeJ mice were immunized ip on weeks 0, 3 and 7 with vaccines containing 40 μg of peptide; sera, obtained 2-3 weeks after each immunization, were tested in an ELISA for IgG antibodies against meningococcal outer membrane proteins. Titers shown are the highest serum dilutions obtained after two or three immunizations which had ELISA values that were (a) more than 0.1 OD unit and (b) twice the value of pre-vaccination sera diluted 1:50.

TABLE 6

ENHANCED SERUM ANTIBODY RESPONSE TO THE GP160 ANTIGENS INDUCED IN RABBITS BY FORMULATING GP160 WITH PROTEOSOMES

Vaccine preparation	Geometric mean of serum IgG titers		
	gp160	gp41	Alex 10*
gp160/alum	30,274	680	1
gp160/proteosome/alum	51,112	565	693

* Alex 10 is a significant epitope of gp120.

Table 7

ELISA Titers of Sera from Mice Immunized with oligo-gp160 formulated with:

A. 50 µg oligo gp160

Sample	No. of doses	Saline control IgG	emulsion IgG	proteos/ saline IgG	proteos/ emulsion IgG	saline control IgA	emulsion IgA	proteos/ saline IgA	proteos/ emulsion IgA
serum	2	256,000	16,000,000	16,000,000	>8,000,000	400	3,200	12,800	12,800
	3	819,000	3,200,000	6,500,000	3,200,000	3,200	25,600	25,600	51,200
vaginal secretions	2	4	512	4,096	16,000	<2	1,024	4,096	16,000
	3	<2	1,024	16,000	128,000	<2	2,048	16,000	64,000
fecal pellets	2	16	32	64	256	4	16	32	32
	3	4	512	4,096	8,192	16	256	512	2,048
intestinal lavage	3	16	8,192	32,000	64,000	16	256	512	1,024
lung lavage	3	512	8,192	16,000	32,000	2	256	512	256

B. 10 µg oligo-gp160

Sample	No. of doses	Saline control IgG	emulsion IgG	proteos/ saline IgG	proteos/ emulsion IgG	saline control IgA	emulsion IgA	proteos/ saline IgA	proteos/ emulsion IgA
serum	2	51,000	4,000,000	8,000,000	16,000,000	100	12,800	3,200	12,800
	3	51,000	3,200,000	4,00,000	13,100,000	800	12,800	6,400	800
vaginal secretions	2	128	256	1,024	16,000	32	256	2,048	16,000
	3	32	1,000	4,026	128,000	32	2,048	2,048	64,000
fecal pellets	2	64	16	16	256	<2	8	16	32
	3	<2	128	512	8,192	32	64	256	2,048
intestinal lavage	3	8	8,192	16,000	16,000	<2	256	128	512
lung lavage	3	4	16,000	8,192	8,192	<2	128	128	256

Table 8

IgG and IgA Antibody Specific Activity for oligo-gp160(451)in Serum and Mucosal Washes of Mice Immunized Subcutaneously or Intranasally with Oligomeric gp160[a]

Group #	Serum		Vaginal		Lung		Intestinal		Fecal	
	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA	IgG	IgA
Subcutaneous										
Ras3C	359	4	<0.7	<0.7	<1.5	-	<0.6	<0.03	<1.6	<0.01
Ras3C	4630	3	<1.0	<1.2	-	-	<2.6	<0.02	<7.1	<0.01
Ras3C	5920	13	<6.7	<4.3	-	-	<2.6	<0.02	<7.1	<0.01
Intranasal										
saline-50	760	5	4	<0.7	41	57	3,560	0.1	751	1.9
saline-15	41	1	77	***24*** [b]	-	-	36	<0.02	76	0.1
prot-50	1,800	24	***30,300***	***9,510***	***12,800***	***552***	5,070*	***195***	***25,100***	2.4
prot-15	3,610	8	7,470*	***2,650***	13,400*	***216***	***138,000***	3.7	3,750	1.4
prot/emul-50	2,400	22	***453,000***	***61,000***	***74,600***	***460***	***89,800***	***147***	***69,600***	13.7
prot/emul-15	1,620	4	***350,000***	***22,700***	***7,910***	***315***	***12,000***	***74***	***17,400***	3.2

[a] Specific activities were calculated by dividing the specific anti-o-gp160 endpoint titer by the total Ig concentration (μg/ml) in each wash or serum. Dashed lines indicate no detectable Ig within the wash. VG refers to vaginal fluids. LG refers to lung fluids. IN refers to intestinal fluids. FE refers to fecal extracts. The following mean total IgG and IgA concentrations were determined: serum IgG (2569±1892); serum IgA (841±399); VG IgG (0.48±0.34); VG IgA (1.14±0.52); LG IgG (0.53±0.48) LG IgA (0.54±0.69); IN (0.60±1.31); IN IgA (33.9±17.2); FE IgG (0.18±0.13); FE IgA (158±47).

[b] Values shown in bold and italic represent an increase of at least 5-fold and values marked by an * represent an increase of between 2- and 5-fold, in specific activity compared to serum. Increases of this magnitude in local or regional IgG or IgA production serve as proof that the antibodies are produced locally and are not a result of serum transudation or blood contamination during preparation of the vaginal, intestinal, lung or fecal material.

All documents cited above are herein incorporated by reference in their entirety, whether specifically incorporated or not.

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations, and conditions without departing from the spirit and scope of the invention and without undue experimentation.

While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A vaccine composition capable of eliciting neutralizing antibodies in a subject to a pathogenic organism which antibodies are present in vaginal secretions, intestinal secretions, lung secretions or feces, which composition comprises:

(a) an antigen comprising a protein or peptide having

(i) an endogenous hydrophobic sequence of between about 3 and about 50 non-polar or uncharged amino acids;

(ii) added to the protein or peptide, an exogenous hydrophobic material comprising a sequence of between about 3 and about 50 non-polar or uncharged amino acids or a C8-C18 fatty acyl group; or

(iii) both (i) and (ii),

(b) complexed with said antigen, a composition comprising proteosomes, bioadhesive nanoemulsions, or both,

wherein said complexed or coupled protein or peptide maintains a native structure of antigenic epitopes such that, upon administration to said subject, the antigen induces neutralizing antibodies in one or more of vaginal secretions, intestinal secretions, lung secretions and feces, capable of neutralizing said pathogenic organism.

2. A vaccine composition according to claim 1 wherein the endogenous hydrophobic sequence or the exogenous hydrophobic material is a sequence of about 5 to about 29 amino acids.

3. A vaccine composition according to claim 1 wherein the exogenous hydrophobic material is a C8-C18 fatty acyl group.

4. A vaccine composition according to claim 3 wherein the exogenous hydrophobic material is lauroyl.

5. A vaccine composition according to claim 1 wherein the exogenous hydrophobic material is Phe Leu Leu Ala Val or Val-Ala-Leu-Leu-Phe.

6. A vaccine composition according to claim 1 wherein the antigen is a is a peptide or peptide oligomer.

7. A vaccine composition according to claim 1 wherein the protein is a viral envelope protein

8. A vaccine composition according to claim 5 wherein the viral envelope protein is an oligomeric gp160 from human immunodeficiency virus.

9. A vaccine composition according to claim 8 wherein said oligomeric gp160 has the sequence of residues 33-681 of SEQ ID NO:1.

10. A vaccine composition according to claim 1 wherein the protein or peptide is recombinantly produced.

11. A vaccine composition according to claim 1 wherein the antigenic protein or peptide natively contains at least one cysteine residue or has at least one added cysteine residue.

12. A vaccine composition according to claim 1 wherein the proteosomes are hydrophobic, multimolecular membrane proteins

13. A vaccine composition according to claim 1 formed by:

(a) bonding the hydrophobic material to said protein or peptide to form a hydrophobic-hydrophilic compound; and

(b) admixing said compound with said proteosomes, bioadhesive nanoemulsions, or both such that said antigen is complexed with said proteosomes or nanoemulsion.

14. A vaccine composition according to claim 13 wherein said admixing step is performed in the presence of a detergent, and is followed by the step of

(c) removing the detergent by dialysis.

15. A vaccine composition according to claim 13 wherein said admixing step is performed lyophilization.

16. A vaccine composition according to claim 1 formulated for intranasal or respiratory administration.

17. A vaccine composition according to claim 1 wherein the vaccine is in a dosage form suitable for multiple inoculations.

18. A vaccine composition according to claim 1 wherein the pathogenic organism is a causative agent of a mucosally-transmitted or sexually transmitted disease.

19. A process for inducing a neutralizing antibody response in a subject against a pathogenic organism resulting in neutralizing antibodies in one or more of vaginal secretions, intestinal secretions, lung secretions and feces, which process comprises administering to the subject an effective amount of a vaccine composition according to claim 1.

20. A process according to claim 19 wherein the exogenous hydrophobic material of said vaccine composition is a C8-C18 fatty acyl group.

21. A process according to claim 19 wherein the exogenous hydrophobic material of said vaccine composition is lauroyl, Phe Leu Leu Ala Val or Val-Ala-Leu-Leu-Phe.

22. A process according to claim 19 wherein the protein is a viral envelope protein.

23. A process according to claim 22 wherein the viral envelope protein is an oligomeric gp160 from HIV-1.

24. A process according to claim 23 wherein said oligomeric gp160 has the sequence of residues 33-681 of SEQ ID NO:1.

25. A process according to claim 19 wherein the antigen is a peptide or peptide oligomer.

26. A process according to claim 19 wherein the protein or peptide is recombinantly produced.

27. A process according to claim 19, wherein said vaccine composition is formed by

(a) bonding the hydrophobic material to said protein or peptide to form a hydrophobic-hydrophilic compound; and

(b) admixing said compound with said proteosomes, bioadhesive nanoemulsions, or both such that said antigen is complexed with said proteosomes or nanoemulsion.

28. A process according to claim 27 wherein said admixing step is performed in the presence of a detergent, and is followed by the step of

(c) removing the detergent by dialysis.

29. A process according to claim 27 wherein said admixing step is performed lyophilization.

30. A process for inducing a neutralizing antibody response in a subject against a pathogenic organism resulting in neutralizing antibodies in one or more of vaginal secretions, intestinal secretions, lung secretions and feces, which process comprises administering to said subject by intranasal or respiratory route a vaccine composition according to claim 16.

31. A process according to claim 19 wherein the pathogenic organism is a causative agent of a mucosally-transmitted or sexually transmitted disease.

32. A process according to claim 30, wherein the pathogenic organism is a causative agent of a mucosally transmitted or sexually transmitted disease.

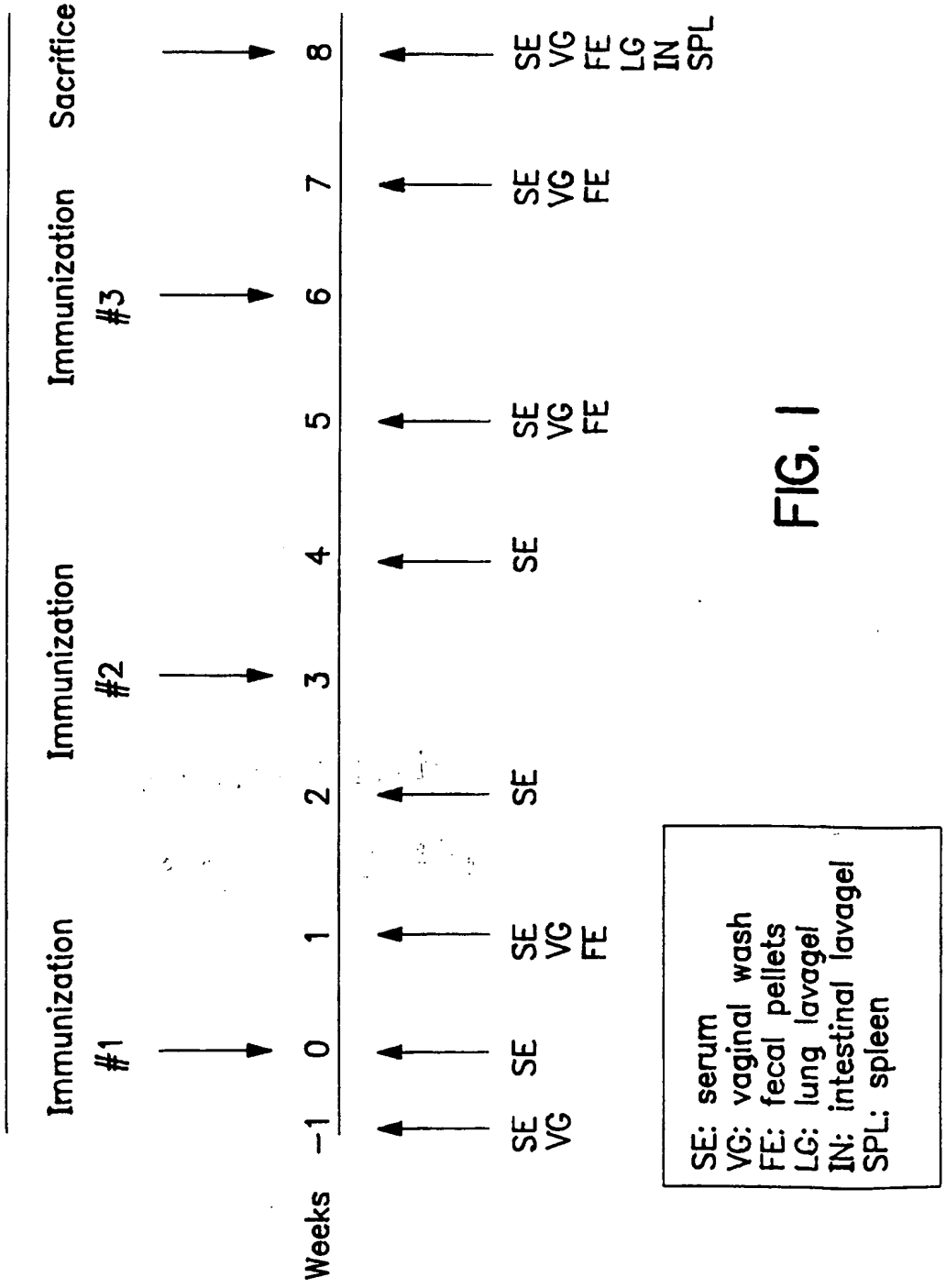

Immunization #1
Immunization #2
Immunization #3
Sacrifice
Weeks
-1
0
1
2
3
4
5
6
7
8
SE VG
SE
SE VG FE
SE
SE
SE VG FE
SE VG FE
SE VG FE LG IN SPL
SE: serum
VG: vaginal wash
FE: fecal pellets
LG: lung lavagel
IN: intestinal lavagel
SPL: spleen

FIG. 1

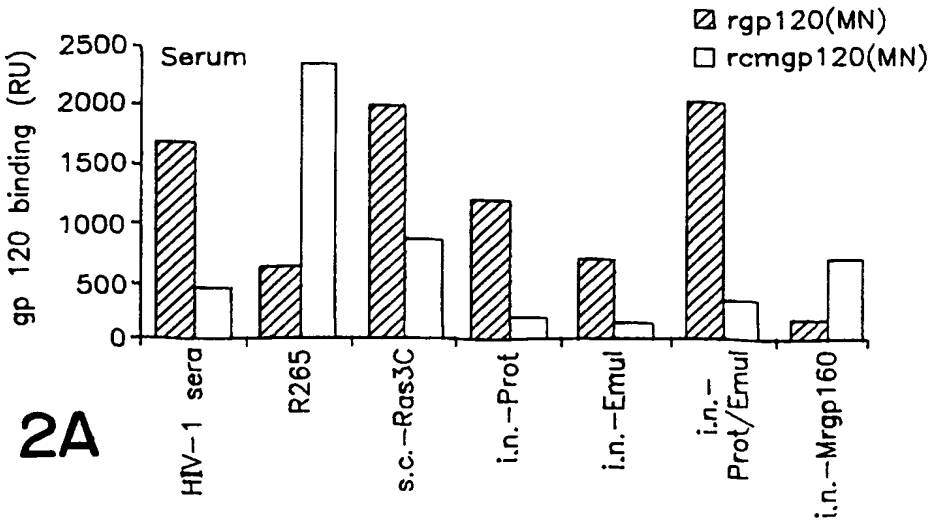


FIG. 2A

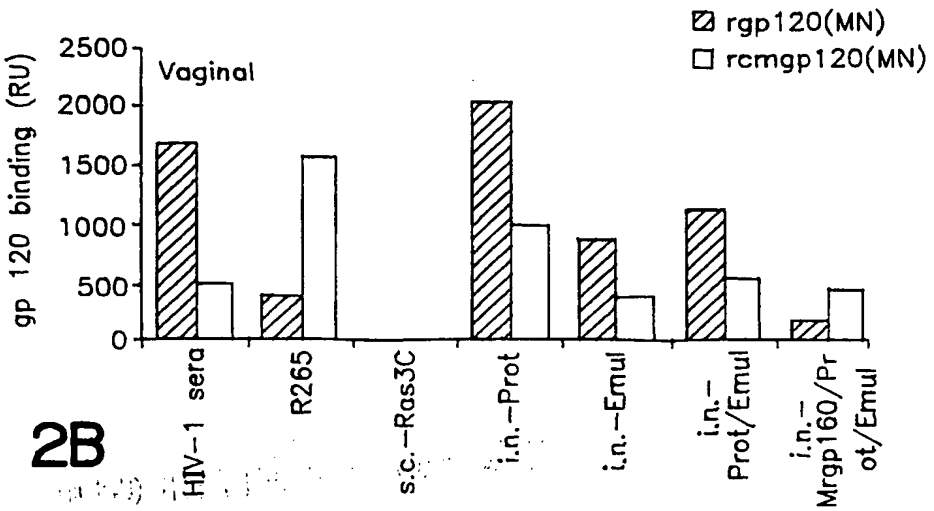


FIG. 2B

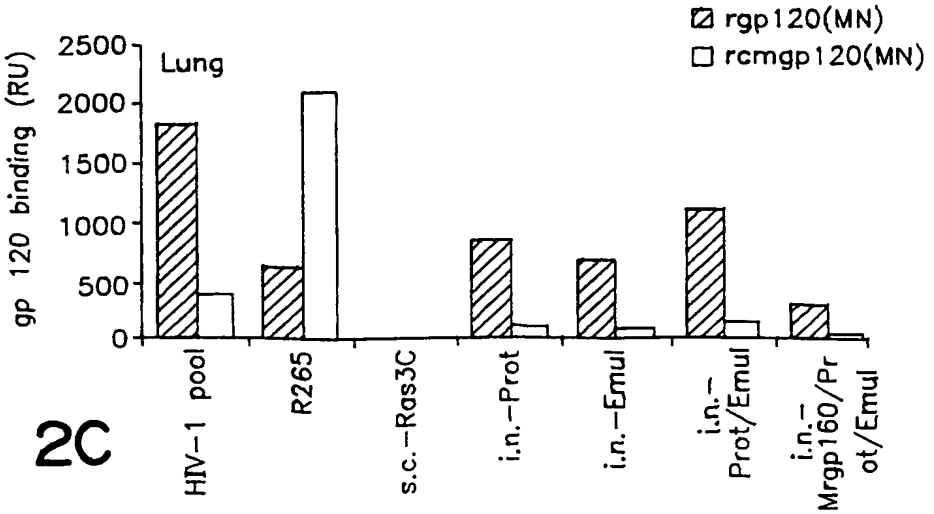


FIG. 2C

FIG. 3A

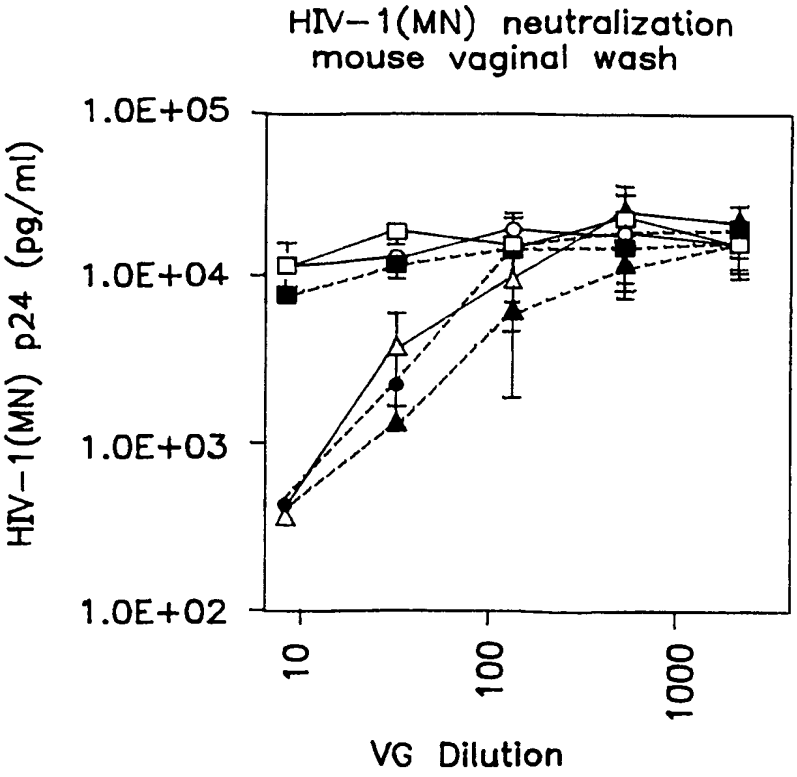

HIV-1(MN) neutralization
mouse vaginal wash
HIV-1(MN) p24 (pg/ml)
1.0E+05
1.0E+04
1.0E+03
1.0E+02
10
100
1000
VG Dilution

FIG. 3B

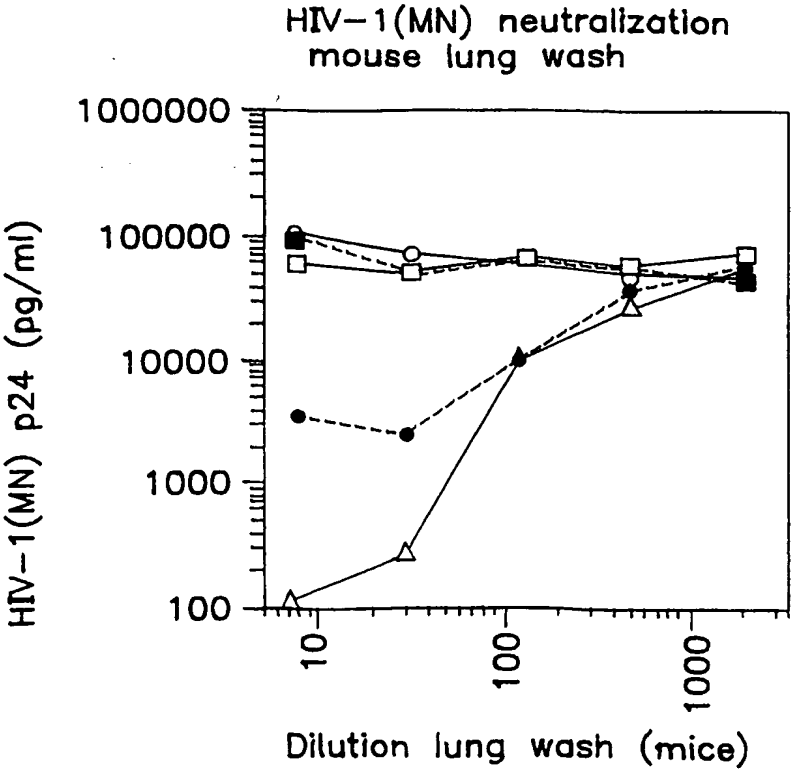

HIV-1(MN) neutralization
mouse lung wash
HIV-1(MN) p24 (pg/ml)
1000000
100000
10000
1000
100
10
100
1000
Dilution lung wash (mice)

FIG. 4A

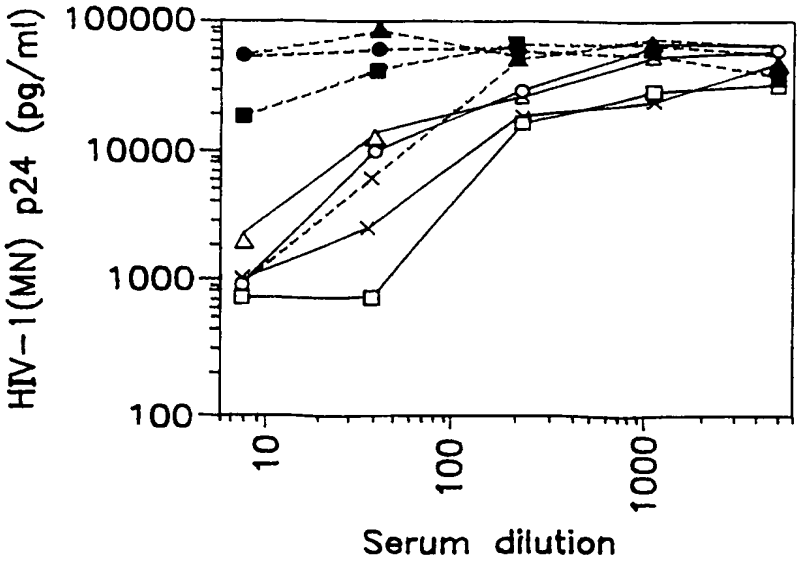


FIG. 4B

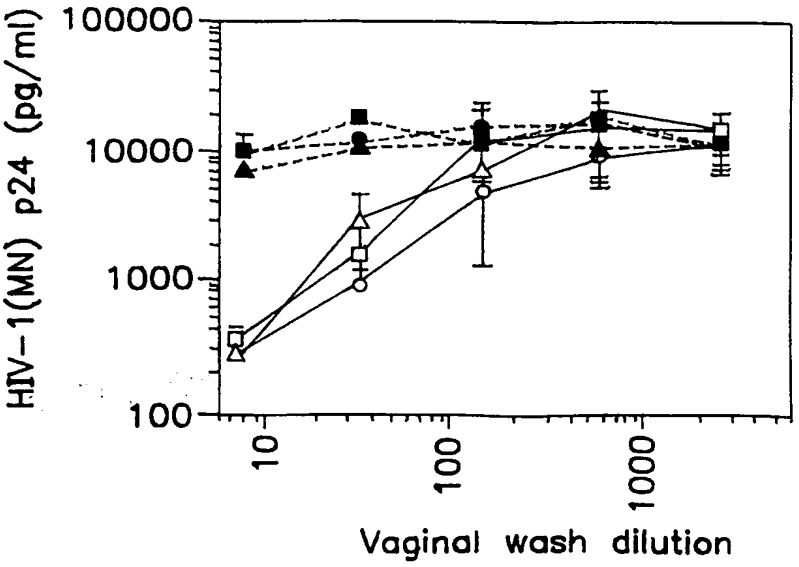
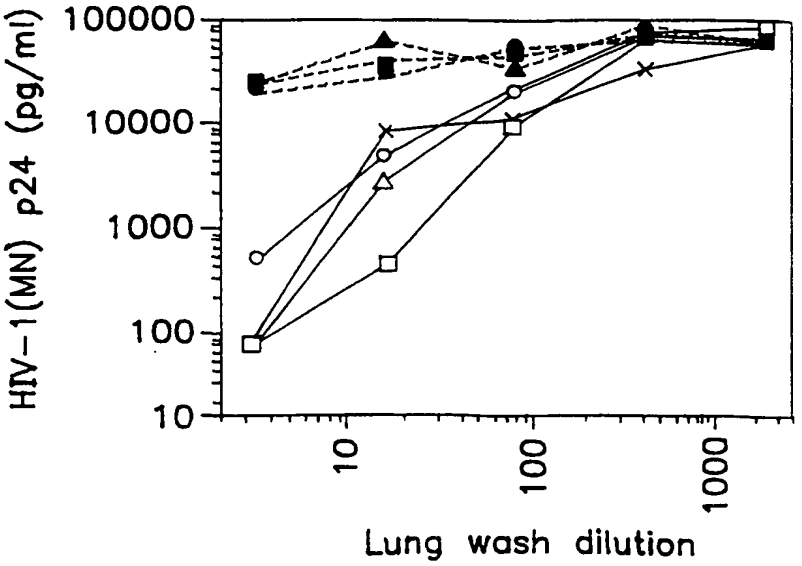


FIG. 4C



PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
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Washington, DC 20231
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in its capacity as elected Office

Date of mailing (day/month/year) 18 March 1998 (18.03.98)	
International application No. PCT/US97/12253	**Applicant's or agent's file reference** 359292000240
International filing date (day/month/year) 10 July 1997 (10.07.97)	**Priority date** (day/month/year) 10 July 1996 (10.07.96)
Applicant LOWELL, George, H. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

15 January 1998 (15.01.98)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Athina Nickitas-Etienne
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